AFRL-SR-AR-TR-10-0331

REPORT DOCUMENTATION PAGE

PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ORGANIZATION.

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
		Jan 2005 - Sep 2008; Dec 2008

4. TITLE AND SUBTITLE

"CELL RESPONSE TO BIOLOGICAL REGULATORS AND NOXIOUS AGENTS: ATTRACTOR STATES AND CONSTRAINED DYNAMICS OF GENE EXPRESSION PROFILES"

5a. CONTRACT NUMBER

FA9550-05-1-0078

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

HUANG, Sui, M.D., Ph.D.

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Children's Hospital Boston and Harvard Medical School
Vascular Biology Program, Karp 11-212
300 Longwood Avenue
Boston, MA

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

AFOSR
Mathematics, Information and Life Sciences
875 North Randolph Street, 4036
Arlington, VA 22203
Dr Walter Kozumbo/NL

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT

Distribution A

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Cells and tissues respond to physiological signals and noxious agents by a coordinated genome-wide change of their gene expression profile which in turn controls the switch between "cellular programs", such as proliferation, differentiation and apoptosis. Characterization of such responses is crucial for civil and defense health purposes (toxicology assessment of novel agents, stem cell control) but typically remains descriptive. No formalism exists to explain dose-response relationship - hampering the understanding of paradoxical effects of low-dose perturbations, such as hormesis. In this project a formal framework was introduced based on two central notions: (1) cell programs, notably the state of differentiation, are attractor states in the high-dimensional state space of genomic regulatory networks, and (2) clonal populations of cells are heterogeneous, hence cells occupy an attractor as a "cloud". Experimental analysis in multipotent cells revealed that heterogeneity is due to slow transcriptome fluctuations within attractors, generating. metastable "outlier" cells that are differentially "primed" to respond to particular perturbations. This explains their qualitatively distinct responses to low doses and provides a unifying concept for multipotency of stem cells. The results will benefit efforts in reprogramming stem cells and in the analysis of the differential effect of varying doses of drugs and toxins on cell behavior.

15. SUBJECT TERMS

gene expression state space; gene expression profile; attractor states; non-genetic heterogeneity; dose-reponse; multipotency, cell fate decision; differentiation; bifurcation; hormesis;

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Sui HUANG
			UU	12	19b. TELEPHONE NUMBER *(Include area code)* 403-210-7858

FINAL PERFORMANCE REPORT

Air Force Office of Scientific Research

Program:
Biological Response Profiling and Assessment

Grant: F49550-05-1-0078

Period covered (including no-cost-extension):
January 1, 2005 – Sept 30, 2008

Grant Title:

"CELL RESPONSE TO BIOLOGICAL REGULATORS AND NOXIOUS AGENTS: ATTRACTOR STATES AND CONSTRAINED DYNAMICS OF GENE EXPRESSION PROFILES"

Principal Investigator:
Sui Huang, M.D., Ph.D.
Vascular Biology Program, Department of Surgery
Children's Hospital
300 Longwood Avenue
Boston, MA 02115

Current Address:
Institute for Biocomplexity and Informatics
Biological Sciences Bldg, Rm 539D
University of Calgary
2500 University Drive NW
Calgary, Alberta T2N 1N4,Canada
Phone: (403) 210-7858
email: sui.huang@ucalgary.ca

1. BROADER CONTEXT OF THE PROJECT: LINK TO PREVIOUS AFOSR GRANT

Given the previous support of this Principal Investigator (PI) by the AFOSR through the program "***Bio-Inspired Concepts***" (hereafter, BIC), BAA 2001-5, grant: F49620-01-1-0564, years **2001-2004**, a brief rather encompassing outline that explains the link between this previous grant and the current grant reported here (Program "***Biological Response Profiling and Assessment***, years **2005-2008**) may be warranted to illustrate the scientific advance at a longer time scale enabled by the continuous support from the AFOSR: The *overarching scientific objective* of both grants is to better understand how cells respond to external perturbations, such as growth or differentiation stimuli or toxins, by combining theories of complex systems with experimental analysis using burgeoning molecular profiling technologies. Thus, in the preceding BIC grant the PI has established *tools* which stimulated and enabled the current project. These tools are both of conceptual and computational nature and were critical for design of experiments and analysis of genome-wide gene expression profile dynamics in the current grant.

First, ***conceptually***, the formal idea that *cell states* (or "genetic programs" of the cell, or "cell fates") correspond to *attractor state* in the dynamics of the gene regulatory network that governs cell fate behavior was examined and for the first time experimentally verified (published in Huang et al, *Phys. Rev. Lett*, 2005 and reviewed in Huang and Kauffman, *Enc. Complexity* – 2009 – see section 5). This led to a novel conceptual framework that helped to bring to a new level our understanding of cellular response to external perturbations that can trigger cell fate switches.

Second, at the ***computational*** front, the development of a computer program, GEDI, that allows for the integrated analysis and display of the high-dimensional state space trajectories (as defined by the temporal evolution of gene expression profiles across the genome) was crucial. It paved the way for a new intuitive understanding of high-dimensional profiles of cellular responses. The algorithm and display method underlying GEDI, has been issued a patent. The figures 1 and 3 exhibit examples of GEDI maps. For details on GEDI see publication Guo et al, *J. Biomed Biotechnol*, 2006 – see section 5)

These conceptual and computational tools established through the BIC grant (2001-2004) provided a novel and unique platform for addressing fundamental as well as pragmatic questions regarding cellular response profiles that goes beyond pure description. They were pivotal for the discovery of further principles in the PI's second AFOSR grant (2005-2008) to which this final report pertains. Specifically, the concept of cell attractors opened a new perspective that stimulated questions concerning the ***detailed dynamics*** of cell phenotype switch during differentiation (=attractor transition) and the ***fate decision*** in multipotent (stem) cells between alternative cell fates. But more practically, the concepts and computational tool were instrumental for the study of ***dose-response relationship*** of cellular high-dimensional molecular responses to external perturbations (see below).

Thus, the current project, albeit technically and formally an independent grant, is scientifically a natural continuation of a line of research of this PI that has been supported. by AFOSR over the past 8 years. The joint results of both projects are of central relevance for efforts in defense-related biological research since they establish a formal framework for the quantitative understanding of the response of gene networks in cell and tissue to a wide range of perturbations, including therapeutic genetic reprogramming and novel or unknown noxious agents in toxicology.

Finally, it is worth mentioning that the process of defining the research goals for this grant was in part shaped by the annual meetings with AFOSR program managers in the BIC program. In particular, the idea to focus on the elementary question of dose-response stem from the discussions about the unmet need to better understand the principles underlying hormesis (see below) stimulated by the program manager at AFOSR.

2. OVERALL GOAL AND SPECIFIC AIMS

2.1. Original objectives proposed in the grant application of 2004:

The overall goal was and is still to better understand the gene expression profile response of cells to perturbations within the framework of high-dimensional attractors. The latter constrains the integrated dynamics of the cell's molecular profile response, allowing for certain some predictions. The three Specific Aims to be pursued to achieve this goal were as follows:

Specific Aim 1: To determine in more detail the structure of the "basin of attraction" of the neutrophilic differentiation state in HL60 cells and to characterize the dynamics for the state transition from the precursor to the differentiated state.

Specific Aim 2: To characterize the biological effect of nanoparticles on endothelial cells and determine if there are gene expression profile signatures that are either pathognomonic of endothelial cell response to nanoparticles or overlap with known profiles of rather "generically" activated endothelial cells.

Specific Aim 3: To identify an experimental in vitro system exhibiting a hormesis response that is amenable to gene expression profiling and other molecular analysis

In the course of the research, these Aims were redefined as a reaction to novel findings on the nature of cell behavior and to keep the investigations focused and realizable as explained in 2.2.

2.2. Two re-defined Objectives

As mentioned in annual progress reports the above original Specific Aims of the grant proposal were modified for reasons and in ways detailed below.

The realization, based on early pilot experiments, that the **(non-genetic) cellular heterogeneity** in clonal and apparently uniform mammalian cell populations may profoundly affect the "macroscopic" biological response entailed a new focus on the phenomenon of non-genetic heterogeneity in the context of attractors (*Specific Aim 1*), while questioning the relevance of monitoring cell response to nanoparticles in the highly heterogeneous endothelial cells (*Specific Aim 2*). Hence, *Specific Aim 2* (see above) was eliminated. In contrary, the non-genetic heterogeneity, a neglected phenomenon in mammalian cell biology, now suggests that low-dose perturbations must be re-examined at the individual cell level – an insight that is of high relevance for studying the phenomenon of hormesis (*Specific Aim 3*).

Hence the research was reorganized during the first half of the funding period of this grant by refocusing on *Specific Aims 1* and *3*. The research was redefined and framed under these two new Objectives: (*A* and *B*), as alluded to in the annual report of 2006 :

Objective A. *To characterize the genomic state space trajectories during differentiation and bi-lineage fate decision.* This effort is directly related to *Aim1* of the original proposal but later also took into consideration cell heterogeneity

Objective B. *To characterize non-genetic heterogeneity in mammalian cell populations, its underlying dynamics and its impact on dose-response relationship for pharmacological perturbations and cell fate decisions.* This objective was stimulated by initial results from *Specific Aim 1.* (Differential response in attractor transition rate in sub-fractions of a cell population after low-dose stimulation) but mostly was inspired by the broader goal to understand fundamental principles of dose-response systems, as originally proposed in *Specific Aim 3.*

3. RESULTS: New conceptual Insights on fundamental properties of cell response

All major new insights of practical significance have been published as original articles in peer-reviewed journals. Specific large scale experimental data are being or have been submitted to public (NIH-supported) databases (see section 5). Below the key findings are summarized and associated relevant publications indicated.

3.1. Summary of new conceptual framework derived from the key findings

The work supported by the present grant led to the erection of the following formal framework that may, with further refinement, lead to a unifying theory for the dynamical behavior of the molecular regulatory network of mammalian cells in response to perturbations:

*The ubiquitous heterogeneity of clonal populations of cells ("**non-genetic heterogeneity**") is neither due to static cell-to-cell variability nor simply a manifestation of fast fluctuations due to "gene expression noise" in individual cells. Rather it arises to a great extent from **slow fluctuations** of gene expression that affect genes across the entire transcriptome. These slow fluctuations appear stochastic but exhibit gene-gene correlation due to the interactions gene regulatory network. They may reflect the dynamics of a complex system on a rugged epigenetic landscape under the influence of stochastic events at multiple levels of regulation.*

*The fluctuations allow individual cells to "scan" the gene expression state space within a restricted region (basin of **attraction**), establishing metastable states with differential responsiveness to particular perturbations for individual cells - depending on the cell's state space position within the attractor. In the case of multipotent stem or progenitor cells this heterogeneity is the basis for **multipotency**. "Outlier cells" at the border of attractors whose fluctuating expression pattern happens to come close to that of a prospective cell fate are reversibly and transiently **primed** for that particular cell fate.*

Corollary: The increased sensitivity of some outlier cells to certain pharmacological/toxicological perturbations at low doses may place them into specific metastable states that may provide, upon additional perturbations, access to particular attractor states not accessible for the bulk of cells when responding to large perturbations.

It is obvious how from this conceptual framework specific hypotheses (corollaries) may be derived to explain many counterintuitive phenomena, including the qualitatively differential response to low vs. high dose perturbations of cellular states (= hormesis).

3.2. Summary of core experimental methodology used

Cell culture and measurements. The cellular system used for experiments that examine the dynamics of regulatory networks and cell population heterogeneity need to be robust, exhibit reliable switching between cell phenotypes (differentiation) and clonally expandable in a homogenous environment. (The latter is crucial for the study of non-genetic heterogeneity). Therefore, the investigations were performed on two lines of hematopoietic cells which grow in homogeneous single-cell suspension culture and have served as solid experimental models in cell biology to study cell ate determination: HL60 and EML cells. ***HL60*** cells are a human promyelocytic cell line derived from acute myeloid leukemia. Its ability to terminally differentiate into mature neutrophils in response to several physiological and non-physiological chemicals were exploited in the studies. ***EML*** cells are a murine bone marrow transgenic cell line which maintains a high multipotency with the ability to differentiate into the erythroid lineages in respond to erythropoietin (EPO) and into the myeloid lineages in response to the cytokines G-CSF or GM-CSF.

Cells were stimulated by treatment with appropriate compounds or factors in various schemes and gene expression and time evolution of the transcriptome in cell populations were determined using microarray-based mRNA profiling (Affymetrix GeneChip and Illumina BeadArrays).

The central novelty was the analysis of subfractions of cells in clonal, apparently homogenous cell populations that traditionally have been treated as an uniform macroscopic

mass for biochemical analysis. Subfractions of cell populations were obtained using fluorescent activated cell sorting (FACS) and single cell derived colonies.

For measurements of gene expression at the individual cell level, flow cytometry (Guava Technologies Cytometer; BectonDickinson FACSCalibur) were used to monitor the population distribution cell surface expression of selected proteins and its time evolution. Cell population heterogeneity was also studied by prospective partitioning of an apparently homogenous clonal cell populations using fluorescence-activated cell sorting, FACS (BD FACSAria) with respect to selected cell surface proteins and subsequent analysis. The explicit analysis and modeling of the time evolution of distributions has not been done before but such analyses that focus on the relaxation of "outlier" cells as they reestablish the original distribution, reveal important information on kinetics of the underlying fluctuations.

Data analysis. Data analysis of gene expression profiles was performed using standard microarray analysis software. But visualization of multiple high-dimensional time courses depended on the use of GEDI (Gene Expression Dynamics Inspector), a program developed by the PI's laboratory under the previous AFSOR grant (BIC) precisely for this purpose. GEDI addresses an unmet need stimulated by the conceptually new approach to expression profiling.

3.3. Key insights achieved

*Below, the three central new insights (**a, b, c**) gained are summarized. Approach and concepts are illustrated using self-explanatory schematic displays. Practical implications are briefly outlined.*

*a. Cell differentiation occurs via hidden, intermediate metastable states in the high-dimensional state space. These intermediate states are exposed by sub-maximal stimulation of differentiation which places cells in such states (**FIG. 1**).*

The neutrophil state in HL60 promyelocytic cells corresponds to a high-dimensional attractor state as evidenced by the convergence of to two high-dimensional trajectories (over ~ 3000 state space dimensions in the high-dimensional state space of the genomic regulatory network) following treatment with the neutrophil differentiation inducers, DMSO and all-trans-retinoid acid. A more detailed analysis however reveals that transition from a progenitor state to the differentiated neutrophil state which appears to be an one-step, yes-or-no response to the differentiation stimulator when one variable (CD11b expression) as "reaction coordinate" (state space dimension) is monitored, actually follows a trajectory which traverses intermediate, metastable states. The latter are not evident since differentiation is usually monitored in response to maximal stimulation and along one single reaction coordinate (observed variable, such as a differentiation marker). Submaximal stimulation exposes these intermediate metastable states.

►***Published in:***

Huang, S., Eichler, G., Bar-Yam, Y., and Ingber, D.E. (2005). Cell fates as high-dimensional attractor states of a complex gene regulatory network. ***Physical review letters*** 94, 128701.

Chang, H.H., Oh, P.Y., Ingber, D.E., and Huang, S. (2006). Multistable and multistep dynamics in neutrophil differentiation. ***BMC Cell biology*** 7, 11.

►***Implications:*** *(i)* Almost nothing is known about dose-response curves with respect to a complex cellular response, such as the switch between cellular programs (e.g., proliferation, differentiation or apoptosis) following pharmacological perturbations. Why do not all cells respond "partially" to an (intermediate) dose? What determines which cells respond and which don't? The findings clearly establish that the heterogeneity of response is not due to stochastic events nor to selective exclusion of inherently non-responsive cells. In contrast, cells respond

differentially because of the intrinsic heterogeneity with respect to state space position: due to transcriptome-wide fluctuations of their internal state they are "smeared out" along the reaction coordinate. Hence, cells that macroscopically appear not to response are actually - with respect to the non-observed dimensions - shifted towards the destination attractor but did not reach it (FIG. 1). They are temporarily "stuck" in an intermediate metastable state – hence primed for increased responsiveness for future perturbations, as demonstrated in Chang et al. (2006) – see above.

(*ii*) The results show that intermediate states exist that are defined in non-observed state space dimensions (bottom box in FIG. 1). Following treatment with a low dose of differentiation-inducers that is not sufficient to trigger full differentiation some cells are placed into these intermediate metastable states. They may then be poised to access specific attractor states (e.g., other fates) not observed in response to full dose which cause differentiation in almost all cell – skipping over these intermediate states. This may be the basis for qualitatively distinct if not opposite, cellular response to varying doses of perturbing agent (hormesis).

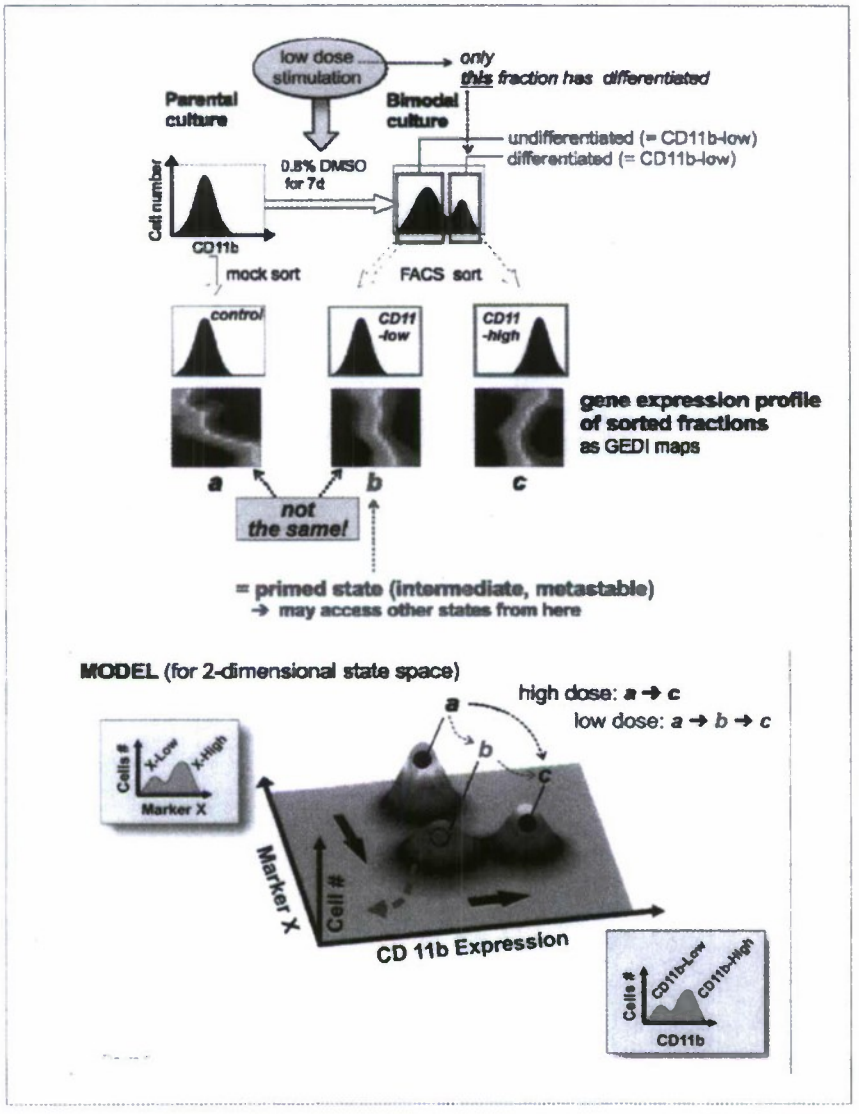

Figure 1. Intermediate metastable state during neutrophil differentiation revealed by low-dose stimulation. Cells are stimulated by low-dose DMSO to partially differentiate, from state a (low CD11b) to state *c* (high CD11b). Box on bottom explains the concepts of hidden dimensions (*x*) not captured by observing a single "reaction coordinate" (differentiation marker, here CD11b). The intermediate state (*b*), although morphologically and in regard to the differentiation marker CD11b indistinguishable from the untreated control cell, actually has a transcriptome that indicates a distinct state – the intermediate, metastable "primed" state not seen in traditional high-dose stimulation. It may be the starting state for qualitatively distinct responses (red dashed arrow) at low concentration of the inducer (hormesis).

b. *Binary cell fate decision in multipotent progenitor cells is initiated by a destabilization of the multipotent cell attractor through a bifurcation event (**FIG. 2**)*

The multipotent state of stem cells or progenitor states is an attractor state. Stimulation to differentiate in various directions (into erythroid or myeloid lineage in EML cells) using distinct differentiation factors (erythropoietin = EPO to trigger erythroid differentiation and GM-CSF to trigger myeloid differentiation) causes first a common change of the transcriptome state despite the opposite developmental paths initiated. This common initial state space trajectory in the high-dimensional state space reflects a destabilization of the progenitor state. This destabilization is mathematically modeled by a ***bifurcation*** in the dynamics of the gene regulatory circuit consisting of the two opposing fate determining transcription factors, GATA1 and PU.1. The bifurcation corresponds, using the classical landscape metaphor, to the conversion of a 'pond' in a valley to a 'watershed' (schematically explained in **FIG. 2**) – hence it places cells to a poised, indeterminacy state between two potential outcomes (downhill movement into the valleys). In fact, once destabilized, subtle bias of gene expression by external signals or stochastic fluctuations can still results in a clear-cut binary decision.

► ***Published in:***

Huang, S., Guo, Y.P., May, G., and Enver, T. (2007). Bifurcation dynamics of cell fate decision in bipotent progenitor cells. ***Dev Biol*** 305, 695-713.

►***Implications:*** The results unite two existing theories of cellular regulation, the longstanding dualism between *selective* and *instructive* regulation. In the former differentiation factors "select" cells that by chance have committed to a particular fate commensurate to the external regulatory signal. Simply said, the differentiation factor such as EPO, thus promote survival and proliferation of cells that spontaneously differentiate and become sensitive to the cognate factor. In instructive regulation the differentiating signal regulates, through specific cellular transduction pathways, precisely the set of genes to be turned on or off such as to generate the gene expression profile of the perspective differentiated cell. Understanding how external factors control cell fate commitment in undecided stem or progenitor cells is of central importance for harnessing the therapeutic potential of stem cells.

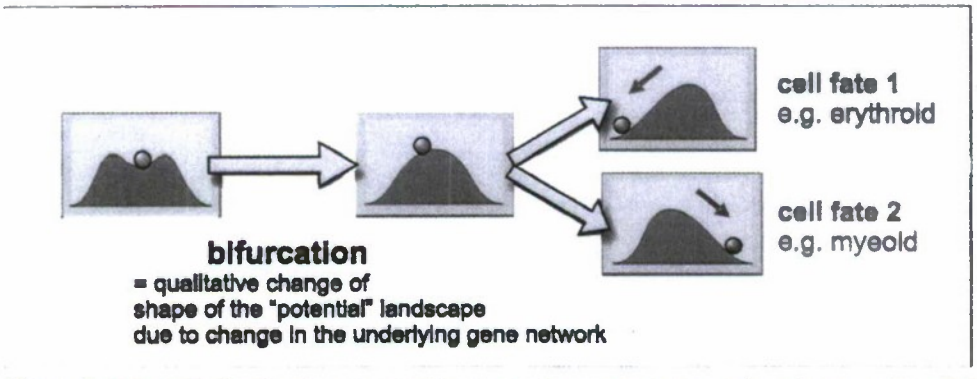

Figure 2. Schematic "cartoon" explaining the binary fate decision by a bifurcation event in which the multipotent progenitor attractor state (central shallow valley) is, upon treatment with differentiating factors converted into a hilltop or watershed, representing an unstable state. The horizontal axis formally represents a state space coordinate and the vertical axis ("elevation") represents the (quasi) potential of every state. After the bifurcation, the fate decision to either fate (red or blue) which is represented by the descent to one of the two valleys on either side of the hill (= attractors of committed lineages) can occur stochastically or be biased by the differentiating signal which would "tilt" the hill. This deterministic model thus must be modified to a stochastic model that takes cell heterogeneity into account (see *3.3c*).

c. Non-genetic heterogeneity in clonal populations of stem cells differentially primes cells for different cell fates (*FIG. 3*)

When monitored at the individual cell level in a cell population, the expression of a particular gene exhibits random fluctuations at various time scales, caused by a variety of processes (among others, "gene expression noise") which lead to the widely observed heterogeneity of clonal cell populations. This heterogeneity is familiarly manifest as a broad *histogram* in flow cytometry (FIG. 1 and FIG 3, top panels) for a particular observable variable, such as the cellular expression level of the stem cell protein *Sca-1* (**FIG 3**). In the formal framework established in the previous AFSOR funded project (see section 1) in which stable cell phenotypes, such as the multipotent cell state, correspond to attractor states of the underlying gene regulatory network, the heterogeneity within a distinct cell type now maps into the *N*-dimensional state space not as a single point but as a "*cloud" of points* within the attractor basin around the attractor state, akin to a swarm of flies around a light source (FIG 3., central panels).

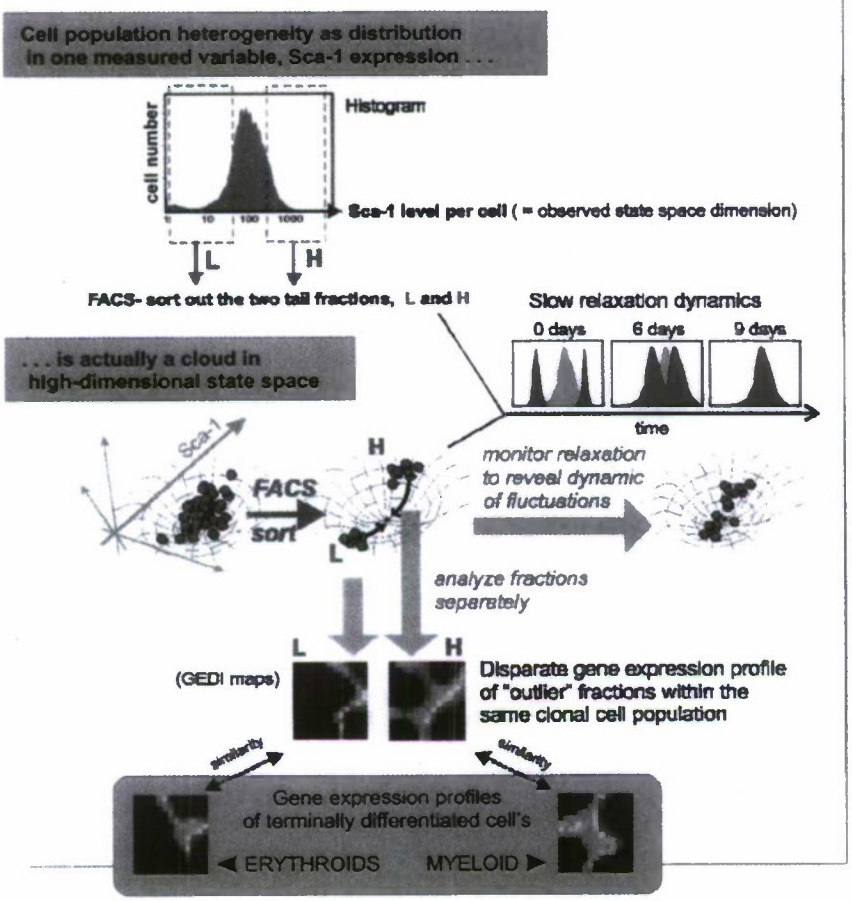

Figure 3. Concept of non-genetic heterogeneity in the high-dimensional state space and experimental approach for its study.
Top panel shows typical flow cytometry histogram of multi-potent EML cells revealing the dispersion of a single trait, here, Sca1-expresion level in individual cells, giving rise to the familiar broad distribution. The novel finding was that the "tail fractions" with the "outlier cells" represent metastable, distinct cell states, as revealed by their gene expression profile (displayed again as GEDI maps below) and the slow relaxation (central panel). The latter was monitored by allowing FACS sorted outlier fractions *H* and *L* (high and low Sca1, respectively) to reconstitute the original distribution which occurred over a period of more than 9 days. This spontaneous relaxation also confirms the attractor character of the cellular state. Interestingly, the gene expression patterns of the outlier cells bear striking similarity with that of the prospective differentiated cell lineages, erythroid and myeloid (bottom). In fact, the Sca-1 *L* and *H* outlier cells are primed to commit to these two cell lineages, respectively (see FIG. 4), although they are still uncommitted multipotent cells which – if not perturbed - will revert back to the center of the attractor due to the slow transcriptome fluctuations

Quantitative, dynamic flow cytometry, cell sorting and single cell colony assays in EML cells in the second half of this grant led to the following conclusions (**FIG 4**):

- In a clonal, apparently homogenous population of stem cells, individual cells exhibited random but *slow* fluctuations of the *entire* transcriptome at a time scale of days-weeks. This indicates that (*i*) individual genes do not fluctuate without any correlation, but, to some extent, the fluctuations are correlated, reflecting the network constraints and that (*ii*) the classical "gene expression noise" is not the sole cause of heterogeneity.
- "*Outlier cells*" of the cloud (still uncommitted cells at opposite borders of the stem cell attractor) were temporally and reversibly "primed" to differentiate into the two opposite available cell fates, in that case, the myeloid and erythroid lineages (**FIG 4a**).
- This differential proclivity is reflected in the fluctuations of the corresponding fate determining factors: Erythroid prone cells had drastically (but transiently) increased expression of the erythroid promoting transcription factor, *GATA1*, where as myeloid prone cells expressed transiently more of the myeloid promoting TF, *PU.1* (**FIG. 4b,c**).

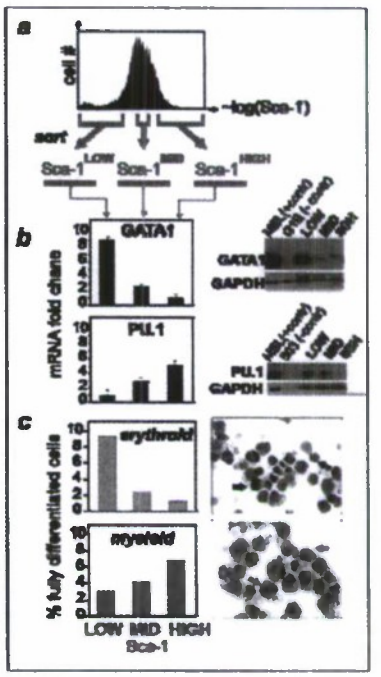

Figure 4. Experimental results showing that the Sca1 outlier cells within a clonal population (panel *a*)are differentially primed to commit to either the erythroid and myeloid lineage (upon treatment with EPO and GM-CSF, respectively, panel c) and that this state of priming is reflected in the spontaneously distinct relative levels of the two opposing fate determining transcription factors, GATA1 and PU.1 (panel *b*).

►Published in:

Chang, H.H., Hemberg, M., Barahona, M., Ingber, D.E., and Huang, S. (2008). Transcriptome-wide noise controls lineage choice in mammalian progenitor cells. ***Nature*** 453, 544-547.

►Implications:

The inevitable heterogeneity in a clonal cell population may be at the very core of the property of multipotency (see BOX under *3.1.*). Thus, each distinct differentiation factor would stimulate only a (transiently) appropriately primed fraction of cells. This finding provides a natural explanation for the notorious inefficacy in attempts to steer multipotent cells stem cells to differentiate into a particular, desired cell lineage – using however complex cocktails of cytokines. A presorting of the stem cell population along some yet to be determined axis of the state space ("priming marker") may increase the efficiency of directed differentiation.

4. SCIENTIFIC PERSONNEL SUPPORTED

Scientists supported over the entire funding period or part of it, and their current position.

- *Sui Huang*, M.D., Ph.D., Principal Investigator
- *Gabriel Eichler*, Graduate Student, bioinformatician (moved on to Boston University/NIH bioinformatics program),
- *Yuchun Guo*, M.S., Computer scientist, software engineer (moved on to MIT graduate program, in Computational Biology)
- *Hannah Chang*, Harvard-MIT-M.D.-Ph.D. student (currently in clinical training of the program)
- *Ying Feng, B.S.* Student, (moved on to Harvard School of Public Health master's program in Bioinformatics/Epidemiology)
- *Amy Brock,* PhD, Postdoctoral fellow, biologist

5. PEER-REVIEWED PUBLICATIONS / DATA DEPOSITED

Publications by the PI during the funding period (2005-2008).
** indicates articles directly related to the project supported by this grant (with some overlap with publications of work initiated by the previous AFOSR grant "BIC" 2001-2004)*

► *Original articles*

1. * Huang S, Eichler G, Bar-Yam Y, Ingber D. Cell fate as a high-dimensional attractor of a complex gene regulatory network. ***Phys. Rev. Lett.*** 94:128701 (2005)
2. Huang S, Brangwynne CP, Parker KK, Ingber DE. Symmetry-breaking in mammalian cell cohort migration during tissue pattern formation: role of random-walk persistence. ***Cell Motil. Cytoskeleton*** 61:201-213 (2005)
3. Barnes CM, Huang S, Kaipainen A et al. Evidence by molecular profiling for a placental origin of hemangioma. ***Proc. Natl. Acad. Sci. U S A*** 102:19097-19102 (2005)
4. * Chang H, Oh P, Ingber DE, Huang S. Multi-stable and multi-step dynamics in neutrophil cell differentiation. ***BMC Cell Biol.*** 7:11 (2006).
5. * Guo, Y, Eichler, GS, Feng Y, Ingber, D, Huang S. Towards a holistic, yet gene-centered analysis of gene expression profiles: a case study of human lung cancers. ***J. Biomed. Biotechnol.*** 2006: 69141 (2006)
6. Kaipainen A, Kieran M, Huang S, Butterfield C, Bielenberg D, Mostoslavsky G, Mulligan R, Folkman J & Panigrahy D. PPAR□ deficiency in inflammatory cell infiltrates suppresses angiogenesis and tumor growth via thrombospondin-1. ***PLoS One*** 2:e260 (2007).
7. * Huang S, Guo YP, May G, Enver T. Bifurcation dynamics in lineage-commitment in bipotent progenitor cells. ***Dev. Biol.*** 305:695-713 (2007).
8. Maliackal JP, Ingber DE, Huang S. Chaotic mean field dynamics of a Boolean network with random connectivity. ***Int. J. Mod. Phys C.*** 18: 1459–1473 (2007)
9. de Bivort B, Huang S, Bar-Yam Y. Empirical multi-scale networks of cellular regulation. ***PLoS Comput Biol.*** 3:1968-78 (2007).
10. Panigrahy D, Kaipainen A, Huang S, Butterfield KE, Barnes CM, Fannon M, Laforme AM, Folkman J, Kieran MW. The PPAR□ agonist fenofibrate suppresses tumor growth through direct and indirect angiogenesis inhibition. ***Proc Natl Acad Sci U S A.*** 105: 985-990 (2008).
11. * Chang H, Hemberg M, Barahona B, Ingber D and Huang S. Transcriptome-wide noise controls lineage choice in mammalian progenitor cells. ***Nature*** 453:544-547 (2008).
12. * Hoffmann M, Chang H, Ingber DE, Huang S, Loeffler M and Galle J. Noise-driven stem cell and progenitor population dynamics. ***PLoS 1.*** 3(8):e2922 (2008)

► ***Reviews:***

1. Huang S and Ingber DE. Cell tension, matrix mechanics and cancer development. ***Cancer Cell*** 8:175-176 (2005)
2. Panigrahy D, Huang S, Kieran MW, Kaipainen A. PPAR□ as a therapeutic target for tumor angiogenesis and metastasis. ***Cancer Biol. Ther.***4: 687-693 (2005)
3. * Huang S, Wikswo PJ. Five dimensions of systems biology. ***Rev Physio, Biochem, and Pharmacol*** 157: 81-104 (2006)
4. * Huang S, Ingber DE. A non-genetic basis for cancer progression and metastasis: self-organizing attractors in cell regulatory networks. ***Breast Disease*** 26:27-54 (2006-2007)
5. * Soneji S, Huang S, Loose M, Donaldson IJ, Patient R, Gottgens B, Enver T, May G. Inference, validation and dynamic modelling of transcription networks in multipotent hematopoietic cells. ***Ann NY Acad Sci.*** 1106: 30-408 (2007)

► ***Book Chapters,***

1. * Huang S, Sultan C, Ingber DE. Tensegrity, Dynamic Networks and Complex Systems Biology: Emergence in Structural and Information Networks within Living Cells. In: Deisboeck TS, Kresh JY and Kepler TB, eds. *Complex Systems Science in Biomedicine*, New York: Kluwer Academic Publishers (2006).

2. * Huang S. Multistability and Multicellularity: Cell Fates as High-dimensional Attractors of Gene Regulatory Networks. In: *Computational Systems Biology*, Eds: Kriete A, Eils R. Elsevier Academic Press (2005)
3. * Ingber DE and Huang S. A complex systems approach to understand how cells control their shape and make cell fate decisions. In: Jorde, LB, Little P, Dunn, M, Subramaniam S, eds. *Encyclopedia of Genetics, Genomics, Proteomics and Bioinformatics* London: John Wiley (2005)
4. * Huang S. and Kauffman S. Gene Regulatory Networks - From Structure to Biological Observable: Cell fate Determination. In: *Encyclopedia of Complexity:* Springer (2009) – in press

► **Gene expression data** obtained are deposited in the NIH/NCBI database (National Institutes of Health, National Center for Biotechnology Information) database for Gene Expression (GEO, Gene Expression Omnibus http://www.ncbi.nlm.nih.gov/geo/)):

For results from 3.1.: Deposition at GEO currently in process.
For results from 3.3.: GEO Series accession number ***GSE10772***.